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Genome-wide association study of circulating Interleukin 6 levels identifies novel loci

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Genome-Wide Association Study of Circulating Interleukin 6 Levels Identifies Novel Loci

Tarunveer S. Ahluwalia^{1,2,#}, Bram P. Prins³, Mohammadreza Abdollahi³, Nicola J. Armstrong⁴, Stella Aslibekyan⁵, Lisa Bain⁶, Barbara Jefferis⁷, Jens Baumert⁸, Marian Beekman⁹, Yoav Ben-Shlomo¹⁰, Joshua C. Bis¹¹, Braxton D. Mitchell¹², Eco deGeus^{13,14}, Graciela E. Delgado¹⁵, Diana Marek¹⁶, Joel Eriksson¹⁷, Eero Kajantie^{18,19}, Stavroula Kanoni²⁰, John P. Kemp^{21,22}, Chen Lu²³, Riccardo E. Marioni^{24,25}, Stela McLachlan²⁶, Yuri Milaneschi²⁷, Ilja M. Nolte³, Alexandros M. Petrelis²⁸, Eleonora Porcu²⁹, Maria Sabater-Lleal^{30,31}, Elnaz Naderi³, Ilkka Seppälä³², Tina Shah³³, Gaurav Singhal³⁴, Marie Standl⁸, Alexander Teumer³⁵, Anbupalam Thalamuthu^{36,37}, Elisabeth Thiering^{8,38}, Stella Trompet^{39,40}, Christie M. Ballantyne⁴¹, Emelia J. Benjamin^{42,43}, Juan P. Casas⁴⁴, Catherine Toben³⁴, George Dedoussis⁴⁵, Joris Deelen^{9,46}, Peter Durda⁴⁷, Jorgen Engmann³³, Mary F. Feitosa⁴⁸, Harald Grallert^{8,49}, Ann Hammarstedt⁵⁰, Sarah E. Harris^{25,51}, Georg Homuth⁵², Jouke-Jan Hottenga^{13,14}, Sirpa Jalkanen^{53,54}, Yalda Jamshidi⁵⁵, Magdalene C. Jawahar³⁴, Tine Jess⁵⁶, Mika Kivimäki⁵⁷, Marcus E. Kleber¹⁵, Jari Lahti^{58,59}, Yongmei Liu⁶⁰, Pedro Marques-Vidal⁶¹, Dan Mellström¹⁷, Simon P. Mooijaart⁴¹, Martina Müller-Nurasyid^{62,63}, Brenda Penninx²⁷, Joana A. Revez⁶, Peter Rossing^{1,64}, Katri Räikkönen⁵⁹, Naveed Sattar⁶⁵, Hubert Scharnagl⁶⁶, Bengt Sennblad^{30,67}, Angela Silveira³⁰, Beate St Pourcain^{22,68,69}, Nicholas J. Timpson²², Julian Troller^{70,71}, CHARGE Inflammation Working Group³, Jenny van Dongen^{13,14}, Diana Van Heemst⁷², Sophie Visvikis-Siest²⁸, Peter Vollenweider⁶¹, Uwe Völker⁵², Melanie Waldenberger⁶², Goncke Willemsen^{13,14}, Delilah Zabaneh⁷³, Richard W. Morris⁷⁴, Donna K. Arnett⁵, Bernhard T. Baune⁷⁵⁻⁷⁷, Dorret I. Boomsma^{13,14}, Yen-Pei C. Chang¹², Ian J. Deary^{25,51}, Panos Deloukas^{20,78}, Johan G. Eriksson^{79,80}, David M. Evans^{21,22}, Manuel A. Ferreira⁶, Tom Gaunt^{81,82}, Vilundur Gudnason^{83,84}, Anders Hamsten³⁰, Joachim Heinrich^{8,85,86}, Aroon Hingorani³³, Steve E. Humphries⁸⁷, J. Wouter Jukema^{39,88}, Wolfgang Koenig⁸⁹, Meena Kumari^{57,90}, Zoltan Kutalik^{16,91}, Deborah A. Lawlor^{81,82}, Terho Lehtimäki³², Winfried März^{15,66,92}, Karen Mather^{70,93}, Silvia Naitza²⁹, Mathias Nauck⁹⁴, Claes Ohlsson¹⁷, Jackie F. Price²⁶, Olli Raitakari^{95,96}, Ken Rice⁹⁷, Perminder S. Sachdev³⁷, Eline Slagboom^{9,46}, Thorkild I.A. Sørensen^{98,99}, Tim Spector¹⁰⁰, David Stacey¹⁰¹, Maria G. Stathopoulou²⁸, Toshiko Tanaka¹⁰², S. Goya Wannamethee⁷, Peter Whincup¹⁰³, Jerome I. Rotter^{104,105}, Abbas Dehghan¹⁰⁶, Eric Boerwinkle^{107,108}, Bruce M. Psaty^{11,109,110}, Harold Snieder³, Behrooz Z. Alizadeh^{3#}

Affiliations

¹Steno Diabetes Center Copenhagen, Gentofte, Denmark

²The Bioinformatics Center, Department of Biology, University of Copenhagen, Copenhagen, Denmark

³Department of Epidemiology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands

⁴Mathematics and Statistics, Murdoch University, Perth, Australia

⁵Department of Epidemiology, University of Alabama at Birmingham School of Public Health, Birmingham, Alabama, USA

⁶QIMR Berghofer Medical Research Institute, Brisbane, 4006, Australia

⁷Department of Primary Care & Population Health, UCL Institute of Epidemiology & Health Care, University College London, London, United Kingdom

⁸Institute of Epidemiology, Helmholtz Zentrum München - German Research Center for Environmental Health, Neuherberg, Germany

⁹Section of Molecular Epidemiology, Department of Biomedical Data Sciences, Leiden University Medical Center, Leiden, The Netherlands

¹⁰Population Health Sciences, University of Bristol, Bristol, United Kingdom

¹¹Cardiovascular Health Research Unit, Department of Medicine, University of Washington, Seattle, WA, USA

¹²Department of Medicine, University of Maryland School of Medicine, Baltimore, MD, USA

¹³Department of Biological Psychology, Behaviour and Movement Sciences, Vrije Universiteit Amsterdam

¹⁴Amsterdam Public Health research institute, Amsterdam University Medical Center, The Netherlands

¹⁵Vth Department of Medicine (Nephrology, Hypertensiology, Rheumatology, Endocrinology, Diabetology), Medical Faculty Mannheim, University of Heidelberg, Mannheim, Germany

¹⁶SIB Swiss Institute of Bioinformatics, Lausanne, Switzerland

¹⁷Centre for Bone and Arthritis Research (CBAR), Department of Internal Medicine and Clinical Nutrition, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

- ¹⁸Chronic Disease Prevention Unit, National Institute for Health and Welfare, PO Box 30, 00271 Helsinki, Finland
- ¹⁹Hospital for Children and Adolescents, Helsinki University Central Hospital and University of Helsinki, Helsinki, Finland
- ²⁰William Harvey Research Institute, Barts & the London Medical School, Queen Mary University of London, London EC1M 6BQ, UK
- ²¹University of Queensland Diamantina Institute, University of Queensland, Brisbane, Queensland, Australia
- ²²MRC Integrative Epidemiology Unit, Population Health Science, Bristol Medical School, University of Bristol, Oakfield House, Oakfield Grove, Bristol BS8 2BN, United Kingdom
- ²³Department of Biostatistics, Boston University School of Public Health, Boston, MA, United States.
- ²⁴Centre for Genomic and Experimental Medicine, Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh, UK, EH4 2XU
- ²⁵Centre for Cognitive Ageing and Cognitive Epidemiology, University of Edinburgh, Edinburgh, UK, EH8 9JZ
- ²⁶Usher Institute of Population Health Sciences and Informatics, University of Edinburgh, Edinburgh, United Kingdom
- ²⁷Department of Psychiatry, Amsterdam UMC, Vrije Universiteit, Amsterdam, The Netherlands
- ²⁸Université de Lorraine, Inserm, IGE-PCV, 54000, Nancy, France
- ²⁹Istituto di Ricerca Genetica e Biomedica, Consiglio Nazionale delle Ricerche, Cagliari, Italy
- ³⁰Cardiovascular Medicine, Department of Medicine Solna, Center for Molecular Medicine, Karolinska Institutet, Stockholm, Sweden
- ³¹Unit of Genomics of Complex Diseases, Institut d'Investigació Biomèdica Sant Pau (IIB-Sant Pau), Barcelona, Spain
- ³²Department of Clinical Chemistry, Fimlab Laboratories, and Finnish Cardiovascular Research Center - Tampere, Faculty of Medicine and Health Technology, Tampere University, Tampere 33520, Finland
- ³³Institute of Cardiovascular Science, University College London, London, United Kingdom
- ³⁴Discipline of Psychiatry, Adelaide Medical School, University of Adelaide, Adelaide Australia
- ³⁵Institute for Community Medicine, University Medicine Greifswald, Greifswald, Germany
- ³⁶Centre for Healthy Brain Ageing, School of Psychiatry, University of New South Wales, Sydney, Australia
- ³⁷Neuropsychiatric Institute, Prince of Wales Hospital, Sydney, Australia
- ³⁸Ludwig-Maximilians-University of Munich, Dr. von Hauner Children's Hospital, Division of Metabolic Diseases and Nutritional Medicine, Munich, Germany
- ³⁹Department of Cardiology, Leiden University Medical Center, Leiden, The Netherlands.
- ⁴⁰Section of Gerontology and Geriatrics, department of Internal Medicine, Leiden University Medical Center, Leiden, the Netherlands
- ⁴¹Baylor College of Medicine, 6565 Fannin Street, MS A-601, Houston, TX 77030
- ⁴²National Heart, Lung, and Blood Institute's and Boston University's Framingham Heart Study, Framingham, MA, United States

- ⁴³ Section of Cardiovascular Medicine and Preventive Medicine, Department of Medicine, Boston University School of Medicine, Boston, MA, United States
- ⁴⁴ Massachusetts Veterans Epidemiology Research and Information Center (MAVERIC), VA Boston Healthcare System, Boston, Massachusetts, USA
- ⁴⁵ Department of Nutrition-Dietetics/Harokopio University, Athens, Greece
- ⁴⁶ Max Planck Institute for Biology of Ageing, Cologne, Germany
- ⁴⁷ Department of Pathology, University of Vermont College of Medicine, Burlington, VT, USA
- ⁴⁸ Division of Statistical Genomics, Department of Genetics, Washington University School of Medicine, St. Louis, MO, USA
- ⁴⁹ German Center for Diabetes Research (DZD), Neuherberg, Germany
- ⁵⁰ The Lundberg Laboratory for Diabetes Research, Department of Molecular and Clinical Medicine, Sahlgrenska Academy at the University of Gothenburg, SE-41345 Gothenburg, Sweden
- ⁵¹ Department of Psychology, University of Edinburgh, Edinburgh, UK, EH8 9JZ
- ⁵² Interfaculty Institute for Genetics and Functional Genomics, University Medicine Greifswald, Greifswald, Germany
- ⁵³ MediCity Research Laboratory, University of Turku, Turku, Finland.
- ⁵⁴ Department of Medical Microbiology and Immunology, University of Turku, Turku, Finland.
- ⁵⁵ Genetics Research Centre, Molecular and Clinical Sciences Institute, St George's University of London, London, United Kingdom
- ⁵⁶ Department of Epidemiology Research, Statens Serum Institut, Denmark
- ⁵⁷ Department of Epidemiology & Public Health, UCL Institute of Epidemiology & Health Care, University College London, London, United Kingdom
- ⁵⁸ Turku Institute for Advanced Studies, University of Turku, Turku, Finland
- ⁵⁹ Department of Psychology and Logopedics, University of Helsinki, Helsinki, Finland
- ⁶⁰ Department of Epidemiology and Prevention, Wake Forest School of Medicine, Winston-Salem, NC, 27157, USA.
- ⁶¹ Department of Internal Medicine, Lausanne University Hospital (CHUV) and University of Lausanne, Rue du Bugnon 46, Lausanne, 1011, Switzerland.
- ⁶² Institute of Genetic Epidemiology, Helmholtz Zentrum München - German Research Center for Environmental Health, Neuherberg, Germany
- ⁶³ Department of Internal Medicine I (Cardiology), Hospital of the Ludwig-Maximilians-University (LMU) Munich, Munich, Germany
- ⁶⁴ Department of Clinical Sciences, University of Copenhagen, Copenhagen, Denmark
- ⁶⁵ BHF Glasgow Cardiovascular Research Centre, Faculty of Medicine, Glasgow, United Kingdom
- ⁶⁶ Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Graz, Austria
- ⁶⁷ Department of Cell and Molecular Biology, National Bioinformatics Infrastructure Sweden, Science for Life Laboratory, Uppsala University, Uppsala, Sweden
- ⁶⁸ Max Planck Institute for Psycholinguistics, Wundtlaan 1, 6525 XD Nijmegen, The Netherlands
- ⁶⁹ Donders Institute for Brain, Cognition and Behaviour, Radboud University, The Netherlands
- ⁷⁰ Centre for Healthy Brain Ageing, School of Psychiatry, University of New South Wales, Sydney, Australia

- ⁷¹Department of Developmental Disability Neuropsychiatry, School of Psychiatry, University of New South Wales, Sydney, Australia
- ⁷²Section of Geriatrics and Gerontology, dept of Internal Medicine, Leiden University Medical Center, Leiden, The Netherlands
- ⁷³University College London Genetics Institute, Department of Genetics, Environment and Evolution, London, UK
- ⁷⁴Department of Population Health Sciences, Bristol Medical School, University of Bristol, Bristol, UK
- ⁷⁵Department of Psychiatry, Melbourne Medical School, The University of Melbourne, Parkville, Australia
- ⁷⁶Department of Psychiatry and Psychotherapy, University of Muenster, Albert Schweitzer-Campus 1, A9, 48149 Muenster, Germany
- ⁷⁷The Florey Institute of Neuroscience and Mental Health, The University of Melbourne, Parkville, Australia
- ⁷⁸Centre for Genomic Health, Queen Mary University of London, London EC1M 6BQ, UK
- ⁷⁹National Institute for Health and Welfare, University of Helsinki, Helsinki, Finland
- ⁸⁰Department of General Practice and Primary health Care, Tukholmankatu 8 B , University of Helsinki, Finland
- ⁸¹MRC Integrative Epidemiology Unit at the University of Bristol, Bristol, BS6 2BN
- ⁸²Population Health Science, Bristol Medical School, University of Bristol
- ⁸³Icelandic Heart Association, Kópavogur, Iceland
- ⁸⁴Faculty of Medicine, University of Iceland, Reykjavik, Iceland
- ⁸⁵Institute and Clinic for Occupational, Social and Environmental Medicine, University Hospital, LMU Munich, Munich, Germany
- ⁸⁶Allergy and Lung Health Unit, Melbourne School of Population and Global Health, The University of Melbourne, Melbourne, Australia
- ⁸⁷Institute of Cardiovascular Science, Rayne Building, University College London, London, UK
- ⁸⁸Durrer Center for Cardiogenetic Research, Amsterdam, The Netherlands
- ⁸⁹Deutsches Herzzentrum München, Technische Universität München, Munich, Germany, DZHK (German Centre for Cardiovascular Research), partner site Munich Heart Alliance, Munich, Germany and Institute of Epidemiology and Medical Biometry, University of Ulm, Ulm, Germany
- ⁹⁰Institute for Social and Economic Research, University of Essex, Colchester, UK
- ⁹¹University Center for Primary Care and Public Health, University of Lausanne, Route de la Corniche 10, Lausanne 1010, Switzerland
- ⁹²SYNLAB Academy, SYNALB Holding Deutschland GmbH, Mannheim, Germany
- ⁹³Neuroscience Research Australia, Sydney Australia
- ⁹⁴Institute of Clinical Chemistry and Laboratory Medicine, University Medicine Greifswald, Greifswald, Germany
- ⁹⁵Centre for Population Health Research, University of Turku and Turku University Hospital; Research Centre of Applied and Preventive Cardiovascular Medicine, University of Turku
- ⁹⁶Department of Clinical Physiology and Nuclear Medicine, Turku University Hospital, Turku, Finland
- ⁹⁷Department of Biostatistics, University of Washington, Seattle, WA, USA
- ⁹⁸Novo Nordisk Foundation Center For Basic Metabolic Research, Section of Metabolic Genetics, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

⁹⁹Department of Public Health, Section on Epidemiology, University of Copenhagen, Copenhagen, Denmark

¹⁰⁰Department of Twin Research and Genetic Epidemiology, King's College London, London, United Kingdom

¹⁰¹MRC/BHF Cardiovascular Epidemiology Unit, Department of Public Health and Primary Care, University of Cambridge, Cambridge, CB1 8RN, UK

¹⁰²Translational Gerontology Branch, National Institute on Aging, Baltimore, MD, USA.

¹⁰³Population Health Research Institute, St George's, University of London, London, United Kingdom

¹⁰⁴Departments of Pediatrics and Medicine, Harbor-UCLA Medical Center, Los Angeles, CA, USA

¹⁰⁵Institute for Translational Genomics and Population Sciences, Departments of Pediatrics and Medicine, Lundquist Institute (formerly LABiomed) at Harbor-UCLA Medical Center, Torrance, CA, USA

¹⁰⁶Department of Epidemiology, Erasmus MC, Rotterdam, The Netherlands

¹⁰⁷Human Genetics Center, School of Public Health, University of Texas Health Science Center at Houston, Houston, TX 77030, USA

¹⁰⁸Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX 77030, USA

¹⁰⁹Departments of Epidemiology and Health Services, University of Washington, Seattle, WA, USA

¹¹⁰Kaiser Permanente Washington Health Research Institute, Seattle, WA, USA.

#Corresponding authors

Tarunveer S. Ahluwalia, PhD, Senior Researcher, Associate Professor, Steno Diabetes Center Copenhagen, Gentofte, and The Bioinformatics Center, Department of Biology, University of Copenhagen, Copenhagen, Denmark
Email: tarun.veer.singh.ahluwalia@regionh.dk

Behrooz Z. Alizadeh, MD, MSc, PhD, Department of Epidemiology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands
Email: b.z.alizadeh@umcg.nl

ABSTRACT

Interleukin-6 (IL-6) is a multifunctional cytokine with both pro- and anti-inflammatory properties with a heritability estimate of up to 61%. The circulating levels of IL-6 in blood have been associated with an increased risk of complex disease pathogenesis. We conducted a two-staged, discovery, and replication meta genome-wide association study (GWAS) of circulating serum IL-6 levels comprising up to 67,428 ($n_{\text{discovery}}=52,654$ and $n_{\text{replication}}=14,774$) individuals of European ancestry. The inverse variance fixed-effects based discovery meta-analysis, followed by replication led to the identification of two independent loci, *IL1F10/IL1RN* rs6734238 on Chromosome (Chr) 2q14, ($p_{\text{combined}}=1.8\times 10^{-11}$), *HLA-DRB1/DRB5* rs660895 on

Chr6p21($p_{combined}=1.5\times 10^{-10}$) in the combined meta-analyses of all samples. We also replicated the *IL6R* rs4537545 locus on Chr1q21 ($p_{combined}=1.2\times 10^{-122}$).

Our study identifies novel loci for circulating IL-6 levels uncovering new immunological and inflammatory pathways that may influence IL-6 pathobiology.

UNCORRECTED MANUSCRIPT

INTRODUCTION

Interleukin-6 (IL-6) is a multifunctional cytokine, which is involved in a wide range of immunomodulatory processes, from cellular migration and adhesion to proliferation and maturation (1, 2). Interleukins are involved in immune cell differentiation and activation (3). IL-6 is synthesized by a variety of different immune cells such as monocytes (4), B-cells (5) and T-cells (6) and also non-immune cells such as epithelial and smooth muscle cells (7), adipocytes (8), endothelial cells (9), and osteoblasts (10).

Several factors have been implicated in circulating IL-6 levels. We have previously demonstrated that IL-6 levels decrease with age in children and increase with age in adults (11). Also, increased levels of IL-6 have been observed in various diseases, not surprisingly in autoimmune diseases such as rheumatoid arthritis (12) and systemic juvenile idiopathic arthritis (13), but also cardio-metabolic diseases like type 2 diabetes (14), heart failure, coronary heart disease (15), and atherosclerosis (16), as well in cancers (17), atopic dermatitis (18), and psychological disorders like depression (19). Due to its implications in the pathogenesis of different disorders, IL-6 has been used as an appropriate choice for drug targeting and used as a monitoring biomarker of disease progression and response to treatments (20). The most illustrious IL-6 inhibitor is tocilizumab (21), a monoclonal antibody binding the IL-6 receptor, which is already in use for treating patients with allergic asthma (22), and immune system disorders like rheumatoid arthritis (23) and systemic juvenile idiopathic arthritis (24), with high efficacy with some initial benefits towards respiratory illnesses like COVID-19 (25).

IL-6 baseline levels are heritable with estimates from twin studies ranging between 15-61% (26-29). However, efforts to identify genetic variants associated

with levels of IL-6 constituted relatively small-scale GWAS (30-33) or sequencing-based candidate gene association studies (34). To date, variants in the IL-6 receptor gene (*IL-6R*) and the gene encoding histo-blood group ABO system transferase (*ABO*) have been identified as statistically significant for an association to IL6-levels. Also, the genetic risk score constructed of *IL-6* variants identified in the study by Shah and colleagues explained up to 2% of the variation in IL-6 levels (33), leaving a substantial part of its heritability unexplained. These seemingly sparse results and limited findings could be due to limitations in the study power caused by low sample size or a great inter-individual variability of IL-6 levels. One may speculate a substantial increase in the study size by increasing the number of participants, which would very likely lead to the identification of additional variants explaining IL-6 levels (35-37).

The current study is the (till date) largest meta GWAS study including 67,428 individuals of European ancestry to identify genetic variants explaining the levels of circulating IL-6 and to understand underlying genetic mechanisms implicated in the pathophysiology of this cytokine.

RESULTS

A total of 52,654 individuals of European descent from 26 cohorts were included in the discovery GWAS meta-analysis with up to 2,454,025 autosomal SNPs passing quality control. Four cohorts (ALSPAC, MONICA/KORA, NTR, and SardinIA), identified genome-wide significant associations in the *ABO* region, whereas none of the other 22 cohorts did, either individually or combined. These cohorts conditioned their results on their relevant top-SNP in *ABO*, the results of which were included in the discovery meta-analyses. The overall genomic control inflation factor (λ_{GC} after correction) at the discovery stage meta-analysis was 1.0.

We identified 94 variants that were genome-wide significantly ($p_{\text{discovery}} < 5.0 \times 10^{-8}$; Supplementary table 1) associated with IL-6 levels, representing two independent genetic loci on chromosomes 1q21 and 6p21. Two common SNPs (rs4537545 and rs660895), one per locus, Chr. 1q21 (*IL6R*), and Chr. 6p21 (*HLA-DRB1/HLA-DRB5*), showed the most significant association with IL-6 levels (index SNPs) and the third SNP (rs6734238) mapped on Chr. 2q14 (*IL1F10/IL1RN*) locus showed suggestive ($5.0 \times 10^{-8} < p_{\text{discovery}} < 1.0 \times 10^{-5}$) association in addition to 5 other loci (*LHFPL3*, *LZTS1*, *GPC5/GPC6*, *USP32/APPBP2*, *STAU1*; Supplementary table 2).

The minor alleles of *IL6R* rs4537545*T ($\beta=0.091$; $p_{\text{discovery}}=8.39 \times 10^{-85}$), *IL1F10/IL1RN* rs6734238*G ($\beta=0.025$; $p_{\text{discovery}}=1.45 \times 10^{-7}$) and *HLA-DRB1/5* rs660895*G ($\beta=0.036$; $p_{\text{discovery}}=1.80 \times 10^{-9}$) associated with increased circulating IL-6 levels (Table 1). Two additional genome-wide significant SNPs in the *IL1R* locus, rs11265618 ($\beta=0.047$; $p_{\text{discovery}}=1.21 \times 10^{-15}$) and rs10796927 ($\beta=0.034$; $p_{\text{discovery}}=1.24 \times 10^{-11}$), in low LD ($r^2 < 0.25$) with the lead SNP rs4537545 were carried

forward for replication, and later conditional analysis as they seemed potential candidates as independent signals.

Overall, 12 SNPs spanning over 9 loci, at a $p_{\text{discovery}} < 1 \times 10^{-5}$ in the discovery GWAS meta-analyses were selected for the replication stage (Supplementary table 2). This included the three index SNPs, two additional SNPs from the 1q21 locus (GWS but in low LD, $r^2 < 0.25$ with index SNP) plus an additional set of seven statistically suggestive SNPs with a p-value of $5 \times 10^{-8} < p < 1 \times 10^{-5}$ in the discovery meta-analyses (either in low LD, $r^2 < 0.25$ with the index SNP or independent loci). Additionally, 3 SNPs as negative controls and 3 SNPs in LD ($r^2 > 0.25$) with the Chr. 1 index SNP, to control for possible genotyping errors of index SNP across replication cohorts, were also added to the replication list, yielding 18 SNPs for replication stage (Supplementary Table 3).

Three loci including Chr.1q21 *IL6R*, Chr.6p21 *HLA-DRB1/5* and Chr.2q14 *ILF10/ILIRN* replicated at $p_{\text{replication}} < 0.05$, reaching GWS; 1q21 rs4537545, $p_{\text{combined}} = 1.20 \times 10^{-122}$; 6p21 rs660895, $p_{\text{combined}} = 1.55 \times 10^{-10}$; and 2q14 rs6734238, $p_{\text{combined}} = 1.84 \times 10^{-11}$ in the combined meta-analyses (Table 1; Supplementary table 3). The two additional signals at Chr.1q21 *IL6R* locus were replicated at $p_{\text{replication}} = 1.7 \times 10^{-4}$ for rs11265618 and $p = 0.03$ for rs10796927, reaching $p_{\text{combined}} = 2.5 \times 10^{-9}$ and $p_{\text{combined}} = 4.1 \times 10^{-13}$, respectively (Supplementary Table 3). The conditional analysis, confirmed that rs11265618 and rs10796927 SNPs were not independent from (Supplementary table 4) but were driven by the index rs4537545 SNP.

In both, discovery and replication association analyses, the effect directionality was generally consistent across individual studies for GWS variants, while there was some evidence of borderline heterogeneity in one of the two novel loci (I^2 (p value) < 0.05) during the discovery and combined meta analysis (Table 1).

The imputation quality scores (r^2) for the GWS (index) SNPs for each cohort (discovery and replication) are available in Supplementary table 5. The other seven SNPs that showed suggestive association in the discovery stage, and expectedly the negative control SNPs did not reach GWS in the combined meta-analyses (Supplementary table 3).

The three GWAS index SNPs when combined, explained approximately 1.06% of the variance in circulating levels of IL-6 using data from the NESDA cohort. The phenotypic variance explained by all the common variants was estimated to be 4.45% using the SumVg method (38).

Replication of other known/suggestive loci for IL-6

IL6R was the only IL6 known locus that we replicate at GWS. *IL1RN* and *HLA-DRB1*, our primary findings have been reported as suggestive loci ($1 \times 10^{-6} < p < 1 \times 10^{-4}$) by Shah *et al.* while some known/suggestive IL6 loci (*ABO*, *BUD13*, *TRIB3*, and *SEZ6L*) did not replicate ($p_{discovery} > 0.05$) in the current study.

SNP functionality

We looked up SNPs in LD with the index SNPs from the immunologically associated loci including *IL-6R*, rs4537545, 1q21; *IL1F10*, *IL1RN*, rs6734238, 2q14, intergenic; and *HLA-DRB1/DRB5*, rs660895, 6p21, intergenic. The search for functional/missense variants in high LD ($r^2 > 0.8$) with the lead SNPs led to the identification of only one nonsynonymous rs2228145 SNP in LD ($r^2 = 0.95$) with the rs4537545 index SNP from the *IL6R* locus. We used the Combined Annotation-Dependent Depletion (CADD) database to identify the functionality, ie. deleterious, disease causal, pathogenicity, of rs2228145 in *IL6R*. CADD is an integrative annotation based on multiple genomic features scored into a single metric (39). *IL6R*

missense the rs2228145 variant has a CADD score of 15.98 (<https://cadd.gs.washington.edu>).

Associations with other traits and gene expression data

Genome-wide significant associations between IL6 associated top SNPs and other traits, and gene expression, data were mined using the Pheno Scanner v2 database (accessed, October 2020).

GWAS based *IL1F10/IL1RN* rs6734238*G allele has been associated with increased levels of serum C reactive protein (CRP) and decreased fibrinogen levels, and blood cell traits in recent GWAS reports (40, 41) (PMID:27863252; Supplementary table 6).

HLA-DRB1/DRB5 rs660895*G allele is associated with increased risk of Rheumatoid arthritis (RA) in Europeans and Asians (42), IgA nephropathy in Asians (43), while the decreased risk of Ulcerative colitis and Inflammatory Bowel Disease (IBD) (44).

IL-6R rs4537545*T allele has been associated with increased circulating CRP levels (45), a decreased risk of RA (42) in mixed ancestries, while an increased risk of diabetes and asthma from the UK Biobank Neale's lab rapid GWAS (See Web Resources; Supplementary table 6). *IL6R* rs4537545T* allele is also associated with C reactive protein, allergic disease, rheumatoid arthritis, and coronary artery disease (Supplementary table 6).

Gene expression: *IL1F10/IL1RN* rs6734238 is associated with *IL1F10/IL1RN* expression levels in the skin, peripheral blood, and whole blood ($p < 5.0 \times 10^{-8}$; Supplementary table 7). *HLA-DRB1/DRB5* rs660895 has been associated with *HLA-DRB1/DRB5/DRB6/DQB1/DQB2* expression levels in multiple tissues including peripheral blood, whole blood, monocytes, adipose tissue, thyroid, tibial artery, coronary artery, heart, lung, brain, colon, skeletal muscle, tibial nerve, skin, and lymphoblastoid cell lines ($p < 5.0 \times 10^{-8}$; Supplementary table 7). *IL6R* rs4537545 SNP

is also associated with IL6R expression levels in peripheral and whole blood ($p < 5.0 \times 10^{-8}$; Supplementary table 7).

Power estimates

Based on power calculator and assumptions mentioned under methods section, the estimated power for the 2 novel index SNPs was 98.3% rs6734238 (Effect allele frequency, EAF: 0.42), and 76.9% rs660895 (EAF: 0.19), respectively.

DISCUSSION

We performed the largest (to date) GWAS meta-analysis for circulating IL-6 levels, which includes 66,341 individuals of European ancestry. We identified three loci associated with levels of circulating of IL-6 in the general population amongst which two are novel (Chr6p21, and Chr2q14), located in/nearby genes (*HLA-DRB1* and *IL1RN/IL-38*) with inflammatory roles explaining up to 1.06 % variance.

The strongest associated SNP, interleukin-6 Receptor (*IL-6R*) rs4537545 at the 1q21 locus, is in high LD ($r^2=0.95$) with a missense *IL-6R* SNP rs2228145 (D358A) that results in an amino acid substitution at position 358 (Asp→Ala) on the extracellular domain of IL-6R and a high CADD score suggesting that the variant is pathogenic or functional or deleterious (among top 10% variants of the genome). The missense SNP is known to impair the responsiveness of cells targeted by IL-6 (46) by reducing IL-6R expression on cell surfaces (47), and increasing levels of soluble IL-6R in individuals homozygous for this mutation (48, 49). Recently it has been demonstrated that increased levels of sIL-6R induced by this variant, can be explained by ectodomain shedding off IL-6R, a mechanism in which membrane-associated proteins are rapidly converted into soluble effectors whereby simultaneously cell surface expression of the same protein is reduced (50). Increased levels of sIL-6R may act as a counter-balance to limit exaggerated IL-6 signaling and may explain the

protective effect of the 358A allele for various cardiovascular diseases including coronary artery disease (CAD) (51-53), atrial fibrillation (54), lung function in asthmatics (55), and abdominal aortic aneurysm (56) as well as RA (57). However in contrast with this finding, the IL-6-sIL-6R complex itself is capable of transducing IL-6 signaling to non-IL-6R expressing cells, known as trans-signaling (58), and it is this mechanism, as opposed to classic signaling, that is linked to chronic inflammatory disorders including IBD and RA (59). Blocking IL-6 signaling cascades can be achieved by using an IL-6R specific inhibitor in the form of a monoclonal antibody, tocilizumab, which is a widely used therapy in the treatment of RA. Several variants in IL-6R, including rs2228145, may assist in the prediction of patient response to tocilizumab in RA (60). The rs4537545*T allele which is associated with IL6 levels is known to associate with increased circulating CRP levels (61) and a decreased risk of RA (42) in studies comprising mixed ancestries. Moreover, this SNP has been associated with IL6R expression in peripheral blood, skin, brain, and adipose tissue (Supplementary table 7). The causal involvement of IL-6 levels in disease remains to be elucidated, but a recent study using a Mendelian randomisation (MR) approach did demonstrate that by using this SNP as instrumental variable, modelling the effects of tocilizumab, that IL-6R signalling has a causal effect on CAD (52). On the other hand, pleiotropic nature of the IL-6R locus, influencing IL-6, CRP, and fibrinogen levels, prohibits instrumental variable analysis and attribution of causality to one particular intermediate. Finally, several other genes encompass the 1q21 locus, including Src Homology 2 Domain Containing E (*SHE*), and Tudor Domain containing 10 (*TDRD10*), but have been ruled out to play a role at this locus (33).

At the identified chromosome 2 locus the lead SNP, rs6734238, is intergenic and has also been associated with circulating CRP and fibrinogen levels (40, 41, 62).

The nearest genes to this locus are the Interleukin 1 Family Member 10 (*IL1F10*, distance=7.6 kB, currently known as *IL-38*) and Interleukin 1 Receptor Antagonist (*IL1RN*, distance=34.4 kB). *IL1F10/IL-38* and *IL1RN* variants (rs6759676 and rs4251961) in partial LD with the lead SNP (r^2_{LD} :0.10 and 0.61) have been recently reported to be protective against the development of insulin resistance (63). This further supports the molecular mechanisms behind IL-6 mediated insulin secretion via glucagon-like peptide 1 (GLP-1) (64) contributing to type 2 diabetes (T2D) pathophysiology. For IL-6 specifically, it has been found that synthesis increases when dendritic cells are stimulated by bacterial lipopolysaccharides (LPS) in the presence of *IL1F10* (65). *IL-1RN* is another member of the interleukin 1 cytokine family, with suggestive evidence for involvement in determining IL-6 levels in the blood. One study found significant associations of *IL-1RN* rs4251961 with plasma CRP and IL-6 levels, albeit not independently replicated and not genome-wide significant ($p=1\times10^{-4}$ and $p=0.004$) (66). Our lead SNP was not in high LD ($r^2<0.8$) with variants in either neighboring genes and therefore in conjunction with its intergenic position, identifying a causal variant in this locus remains non-trivial.

The 6p21 rs660895, which was identified, resides within the HLA region, which forms one of the most complex genomic regions to study due to its large LD blocks and sequence diversity. This region has some population substructure in Europeans which may have influenced the results however 1) each cohort population substructure adjustment was applied, followed by genomic correction for overall discovery stage meta-analyses. Thus, we reduced the chances that the population substructure may have had on this locus. The nearest genes to the index SNP, *HLA-DRB1* (distance=19.8 kB), and *HLA-DQA1* (distance=27.8 kB) are both histocompatibility complex genes encoding proteins that form cell surface complexes

for certain immune system cells helping in antigen presentation to trigger an immune response. It is noteworthy that variations at this locus code for antigen-presenting complexes (APCs) which have been previously associated with diseases having a dysfunctional immune system; while we report for the first time that there exists also a strong association of this locus with circulating cytokine levels. Therefore, the association of this locus with the disease may corroborate through its effect through IL6 levels. One high-LD SNP (rs9272422, $r^2=0.82$ with our index SNP, rs660895) residing in the promoter region of *HLA-DQA1*, support this hypothesis and has been identified previously for Systemic Lupus Erythematosus (67) and Ulcerative Colitis (68). rs660895*G allele is associated with increased risk of RA in Europeans and Asians (42), IgA nephropathy in Asians (43), while the decreased risk of Ulcerative colitis and IBD. (44)

Various studies aimed to identify genetic variation underlying levels of IL-6 (22-26) have found genome-wide significant associations in the *IL-6R* and *ABO* genes. The study performed by Shah and colleagues (33) found suggestive evidence (non-GWS; $p=3.8\times 10^{-6}$ respectively) for additional loci, including *ABO*, *BUD13*, *TRIB3*, and *SEZ6L*, none of which replicate in the current study ($p_{discovery}>0.05$) indicating that these might be false-positive findings due to low sample size (~7800) or loci with sex-specific effects (associations based on women dominant population) or due to technical shortcomings with measurement assay (*ABO* locus).

It is surprising that even with increased statistical power ($n_{discovery}=52,654$; $n_{replicaiton}=14,774$) in the current study (compared to the previous IL6 GWAS) (33), we could identify three genetic loci (1q21, 2q14, and 6p21) accounting for ~ 1% of the genetic variance for circulating IL-6 levels. According to the current estimates, the heritability levels for IL6 levels range between 15 to 61%, suggesting that an

enormous increase in sample sizes would be required to identify additional variants explaining this remaining heritability. Multiple explanations for this so-called missing heritability phenomenon have been proposed in the past, which can be sought in rare or low frequency coding variants as observed for a similar metabolic quantitative trait by us (69) or can be explained by non-additive effects which may cause inflated estimates of heritability. Plausible evidence for other sources of unexplained heritability that have been found are epigenetic changes, and haplotypes of common SNPs.

Collectively, our results provided additional insights into the biology of circulating IL-6. We identify new loci, limited by common variants in the Hap Map Reference panel. Albeit this is comparable to the 1000 genomes reference panel (70) but narrower compared to some newly available panels that show greater variant coverage in numbers and frequency range. Future studies are recommended to aim for identification of additional common but also rare variants, by firstly using richer imputation panels, such as UK10K project or the Haplotype Reference Consortium, a strategy that holds great promise, and secondly by making use of genetically isolated populations. Thirdly, we would like to stress the importance of phenotype harmonization. As we identified genome-wide variants in the *ABO* locus, in four studies participating in the discovery, but not in the remaining 22 cohorts, there is a strong indication that this locus may be assay-specific. However, a proper demonstration of this hypothesis would require further testing, including repeating the GWAS in *ABO* positive cohorts using a different IL-6 assay. Indeed it is emerging that the *ABO* locus has pleiotropic effects on many different traits and diseases (71), which would suggest a more thorough analysis before disregarding this signal. Also, conventionally increasing sample sizes without correction for population

substructures may raise heterogeneity within populations (72), likely concealing the SNPs that affect particular subgroups. Future specific studies should counter the widely held assumption of unconditional risk alleles of complex traits and focus on the importance of studying more homogenous subgroups to, for example, investigate the age-dependent effect of genetic variants (73, 74). Here while further exploring the pleiotropic effect of IL-6-related variants, we identified phenotypes differentially regulated by diverse variants in the 1q21 locus. Biologic systems are dynamic complex networks and are evolving through lifespan and investigating the interrelationships existing between phenotypes as well as between genetic variations and phenotypic variations has the potential for uncovering the complex mechanisms. This is the case here for IL-6 and tailored methodologies should be devoted to the study of such traits, hopefully resulting in clinically significant breakthroughs. Future collaborative efforts therefore should strive to use well-calibrated assays, z-standardized protocols for sample handling, and processing⁷⁵, though this will be difficult to achieve in practice. Lastly, we have attempted to perform formal association based causal analysis to identify the likely causal loci, using the DEPICT approach; unfortunately, instead with only 2 novel GWS findings, our analyses were underpowered and thus not included. We also mine the gene expression and eQTL data for the identified SNPs using established databases, however we were unable control for random co-localization signals or other confounders as we had limited access to summary level data. In conclusion, we identify two novel common genetic variants associated with circulating IL-6 levels that may influence the pathophysiology of complex cardio-metabolic, psychiatric and immunological traits, among individuals of European ancestry. This is a step further towards unravelling new biological pathways and potential therapeutic targets that can be developed for

the IL-6 related disorders, while suggesting looking deeper into the genome for coding variants (rare and common) having larger individual effects.

UNCORRECTED MANUSCRIPT

MATERIAL AND METHODS

Discovery Stage

Study populations

The overall study design (Supplementary figure 1) involved the discovery cohorts with 53,893 individuals. After overlapping individuals with available genotype and phenotype data, the discovery stage included 52,654 individuals from 26 cohorts of European ancestry listed under Supplementary table 8, described in Supplementary text S1 and, study summary characteristics in Supplementary table 9. Only population-based samples or healthy controls from case-control studies were included in the final analyses.

Serum IL-6 measurements

Each study typically collected venous blood samples stored below -80°C until the time of measurement using various types of immunoassays and expressed as pg/ml as presented in Supplementary table 10. The trait transformation and phenotype data quality control (QC) were presented by Supplementary text S3. (Supplementary text S3.1 and S3.2). In brief, participating cohorts have checked for the percentage of missingness in IL6 measurements and evaluated for indices of QC (Supplementary text S3.2), yielded the final number of participants with available validated IL6 levels, of whom those with available genotype data were included in the study as characterized in Supplementary table 9 and in Supplementary text S1 and S2.

Genotyping and imputation

Each participating cohort performed genome-wide genotyping using a variety of genotyping platforms, and applied a predefined quality control (QC) of genotype data (Supplementary table 11) followed by performing imputation of non-genotyped genetic variants, on the backbone of haplotypes inferred from the Hapmap Phase II

reference panel (NCBI Build 36), and using statistical software such as IMPUTE (75), MACH, Minimac (76), or BIMBAM (77) (Supplementary table 11). Each cohort was recommended a set of general SNP quality filters including $MAF < 0.01$; Hardy Wienberg Equilibrium (HWE) $p \leq 10^{-6}$; imputation quality $r^2 \leq 0.3$; and genotyping call rate < 0.95 (Supplementary figure 1). Once we received summary results from each participating study, we ran a series of QC checks. Firstly these included a set standard checks, including the imputation quality filters (basis the imputation program used and/or $r^2_{\text{imputation}} < 0.3$ were excluded), and then checks for genomic inflation (quantile-quantile or QQ plots). We adapted filter thresholds per cohort to reduce any observed deviation from the null while missing SNP loss due to the QC process. Finally ~2.45 million (2,454,025) common SNPs, were part of the discovery meta-analysis.

Statistical methods

GWAS analysis

Each study conducted an independent GWAS analysis between SNPs and natural log-transformed values of serum IL-6 levels following a predefined analysis plan (Supplementary methods S4). Association analyses were conducted using linear regression model, or linear mixed effect models to account for familial correlation when warranted, with additive genetic effects, accounting for imputation uncertainty while adjusting for age, sex, population sub-structure (through study-specific principal components), and/or study-specific site, when necessary. GWAS summary result obtained from each cohort underwent a series of QC checks using the QCGWAS package in R (78) (Supplementary text S3 and Supplementary figure 1). Being aware of the potential false-positive association in the *ABO* region on chromosome 9 (28, 30), while using an R&D systems high-sensitivity assay kit to

measure IL6 levels (R&D systems, Minneapolis, MN, USA), four (out of 22) discovery cohorts that observed genome-wide significant results in the *ABO* locus, were asked to rerun the GWAS analysis conditional on the top *ABO* SNP (ie. rs8176704) before including them in the final discovery meta-analysis (Supplementary text S3.3).

Discovery GWAS meta-analyses

Individual GWAS results from 26 European studies were meta-analyzed using the inverse variance weighted, fixed-effects method as implemented in GWAMA while applying the double genomic control (GC) correction for population stratification i.e. first to each study individually and subsequently also to the pooled results after meta-analysis(79).

Regional association plots for the discovered loci were generated through the LocusZoom (78) tool. We used the SNAP tool (80) to perform the pairwise LD checks (HapMap release 22 data) and to verify low LD with secondary signals. All SNPs selected for the replication stage had to fulfill the following criteria: (i) having an association $p_{discovery} \leq 1 \times 10^{-5}$ and being in very low LD with the index SNP ($r^2 < 0.2$), and (ii) available in at least 50% of study cohorts.

Replication and Combined Meta-analysis

Study population, phenotyping and QC

The overall study (Supplementary figure 1) comprised 15,785 individuals for replication. After removing individuals with missing data, the replication analyses were performed using a combination of *in silico* and *de novo* genotyping in 14,774 individuals from 12 cohorts of European ancestry as described in Supplementary text

S2. Similar QC (Supplementary text S3, and Supplementary table 11) and statistical checks were made as in the discovery stage.

Venous blood samples (serum or plasma) were collected and stored at -80°C. Serum/plasma IL-6 levels (pg/ml) were measured using various immunoassay methods described in Supplementary table 10. Each cohort tested the selected SNPs using the same statistical model as for the discovery association analyses (Supplementary figure 1). Effect size estimates of all replication SNPs from each replication study were compared with the effect size estimates from the discovery meta-analyses. When effect sizes from individual cohorts did not align, we excluded these cohorts from the replication meta-analyses ($n_{\text{cohorts}}=3$). To account for the inter-study assay differences insensitivity, we combined results across the replication studies using a fixed effect sample-size weighted Z-score meta-analysis as implemented in the METAL package (<https://genome.sph.umich.edu/wiki/METAL>) (81).

The summary GWAS meta-analyses result from the discovery and replication stages were then used to perform a combined (discovery + replication) GWAS meta-analysis using a sample-size weighted Z-score method. Test for heterogeneity was also performed as part of the meta-analysis package using METAL where I^2 statistic denoting the percentage of variation across studies was estimated ($I^2 = 100\% \times (Q - df)/Q$) where Q is the Chi-Square statistic. Significance for heterogeneity was denoted by the heterogeneity (or Het_p) p values. Variants that were significant in the replication meta-analysis at $p < 0.05$ and had an overall $p_{\text{combined}} < 5 \times 10^{-8}$ in the combined meta-analysis were considered statistically GWS. SNPs within the range of 1 Mb (or 10^6 bases) on either side of the most significant (ie. index) SNP (with LD,

$r^2 > 0.25$) were considered part of the same locus whereas those in low LD ($r^2 < 0.25$) were tested if they were independent using conditional analysis.

Conditional analysis

We performed an approximate joint conditional analysis to identify distinct signals in a specific chromosomal region as implemented in GCTA (82) using high quality genome-wide genotyped/imputed reference data from two studies (NEtherlands Study of Depression and Anxiety (NESDA) from the Netherlands and/or Genetics of Obesity in Young Adults (GOYA) from Denmark) to estimate linkage disequilibrium (LD) (83) between SNPs.

Conditional analysis for identification of independent signals was performed on GWS SNPs (± 1 Mb to the index SNP and having low LD, $r^2 < 0.2$ with the index SNP) using summary statistics from the discovery GWAS meta-analysis data (--COJO option in GCTA) after confirming the GWS loci from the combined meta-analysis.

Heritability estimates

We approximated the variance explained by all distinct lead SNPs from the meta-analysis using the following formula:

$$\sum_{i=1}^n \frac{\beta_i^2 \cdot 2 \cdot \text{EAF}_i \cdot (1 - \text{EAF}_i)}{\sigma^2(\text{residuals}(\ln(\text{IL6})))}$$

where EAF is the effect allele frequency, and β_i the effect size of the individual variants and n is the total number of lead variants. The current formula may overestimate the variance to a small extent as some level of SNP correlation was existent (LD $r^2 < 0.25$). The variance of the residuals of $\ln(\text{IL-6})$ was calculated using data from the NESDA cohort ($n=2,517$). The total common SNP heritability of serum

IL-6 levels explained by all GWAS variants was estimated using the observed Z-statistics from the discovery analyses for a subset of pruned SNPs. Following the original method (SumVg) (38), we pruned the imputed (based on the 1000G *Phase1 Integrated Release, Version 3, 2012.04.30* reference panel) genotypes of the NESDA cohort using PLINK v1.07 (84), by removing correlated SNPs at $r^2 > 0.25$ within a 100-SNP sliding window, and with a step size of 25 SNPs per forwarding slide. This resulted in a pruned set of 163,459 SNPs.

SNP mapping and functionality

We searched for variants in high LD ($r^2 > 0.8$) within a 1 Mb region on either side of the lead SNPs using 1000 Genomes sequence data (Phase1 Integrated Release, Version 3, 2012.04.30), utilizing tools available in Liftover (85), VCFtools (86), and clumping in PLINK (84). We subsequently annotated these variants using ANNOVAR (87) with the RefSeq (88) database for variant function and genic residence or distance. We used the Combined Annotation-Dependent Depletion (CADD) database to identify the functionality, ie. deleterious, disease causal, pathogenicity, for the index SNPs.

Associations with other traits and gene expression data

PhenoScanner v2 (89) data mining tool was used (Access date October 2020) to identify existing GWS (at $p < 5 \times 10^{-8}$) associations between IL6 identified SNPs and other traits, and gene expression/eQTLs data.

Power calculation

We used GWAs power estimator (see Web Resources) by assuming a relative risk of 1.10, given $N = 66,000$, α (p-value) $= 5 \times 10^{-8}$.

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Conflict of Interest

There is no conflict of interest from any of the participating co-authors. TSA is a shareholder with Novo Nordisk A/S and Zealand Pharma A/S.

Authors Contributions

The guarantors of the study are TSA, BP, and BZA, from conception and design to conduct of the study and acquisition of data, data analysis, and interpretation of data. TSA and BP have written the first draft of the manuscript. All co-authors have provided important intellectual input and contributed considerably to the analyses and interpretation of the data. All authors guarantee that the accuracy and integrity of any part of the work have been appropriately investigated and resolved and all have approved the final version of the manuscript. BP had full access to the data and the corresponding authors TSA and BZA had the final responsibility for the decision to submit for publication.

WEB RESOURCES

QCGWAS, <https://cran.r-project.org/web/packages/QCGWAS/index.html>

GWAMA, <http://www.well.ox.ac.uk/gwama/>

METAL, <http://csg.sph.umich.edu/abecasis/metal/>

GCTA, <http://www.complextraitgenomics.com/software/gcta/>

LocusZoom, <http://csg.sph.umich.edu/locuszoom/>

1000 Genomes, <ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/release/20110521/>

PLINK, <http://pngu.mgh.harvard.edu/~purcell/plink/>

VCFtools, <http://vcftools.sourceforge.net/>

ANNOVAR, <http://www.openbioinformatics.org/annovar/>

PhenoScanner, <http://www.phenoscaner.medschl.cam.ac.uk/phenoscaner>

Power calculations:

http://csg.sph.umich.edu/abecasis/cats/gas_power_calculator/index.html

The UK Biobank Neale's lab rapid GWAS:

(<http://www.nealelab.is/blog/2017/7/19/rapid-gwas-of-thousands-of-phenotypes-for-337000-samples-in-the-uk-biobank>)

REFERENCES

- 1 Kishimoto, T. (2010) IL-6: from its discovery to clinical applications. *Int Immunol*, **22**, 347-352.
- 2 Nishimoto, N. and Kishimoto, T. (2006) Interleukin 6: from bench to bedside. *Nat Clin Pract Rheumatol*, **2**, 619-626.
- 3 Brocker, C., Thompson, D., Matsumoto, A., Nebert, D.W. and Vasiliou, V. (2010) Evolutionary divergence and functions of the human interleukin (IL) gene family. *Hum Genomics*, **5**, 30-55.
- 4 Gelinas, L., Falkenham, A., Oxner, A., Sopel, M. and Legare, J.F. (2011) Highly purified human peripheral blood monocytes produce IL-6 but not TNFalpha in response to angiotensin II. *J Renin Angiotensin Aldosterone Syst*, **12**, 295-303.
- 5 Kitani, A., Hara, M., Hirose, T., Harigai, M., Suzuki, K., Kawakami, M., Kawaguchi, Y., Hidaka, T., Kawagoe, M. and Nakamura, H. (1992) Autostimulatory effects of IL-6 on excessive B cell differentiation in patients with systemic lupus erythematosus: analysis of IL-6 production and IL-6R expression. *Clin Exp Immunol*, **88**, 75-83.
- 6 Li, T. and He, S. (2006) Induction of IL-6 release from human T cells by PAR-1 and PAR-2 agonists. *Immunology and cell biology*, **84**, 461-466.
- 7 Ng, E.K., Panesar, N., Longo, W.E., Shapiro, M.J., Kaminski, D.L., Tolman, K.C. and Mazuski, J.E. (2003) Human intestinal epithelial and smooth muscle cells are potent producers of IL-6. *Mediators Inflamm*, **12**, 3-8.
- 8 Fain, J.N. (2006) Release of interleukins and other inflammatory cytokines by human adipose tissue is enhanced in obesity and primarily due to the nonfat cells. *Vitam Horm*, **74**, 443-477.
- 9 Podor, T.J., Jirik, F.R., Loskutoff, D.J., Carson, D.A. and Lotz, M. (1989) Human endothelial cells produce IL-6. Lack of responses to exogenous IL-6. *Ann N Y Acad Sci*, **557**, 374-385; discussion 386-377.
- 10 Sanchez, C., Gabay, O., Salvat, C., Henrotin, Y.E. and Berenbaum, F. (2009) Mechanical loading highly increases IL-6 production and decreases OPG expression by osteoblasts. *Osteoarthritis Cartilage*, **17**, 473-481.
- 11 Haddy, N., Sass, C., Maumus, S., Marie, B., Driesch, S., Siest, G., Lambert, D. and Visvikis, S. (2005) Biological variations, genetic polymorphisms and familial resemblance of TNF-alpha and IL-6 concentrations: STANISLAS cohort. *Eur J Hum Genet*, **13**, 109-117.
- 12 Madhok, R., Crilly, A., Watson, J. and Capell, H.A. (1993) Serum interleukin 6 levels in rheumatoid arthritis: correlations with clinical and laboratory indices of disease activity. *Ann Rheum Dis*, **52**, 232-234.
- 13 de Benedetti, F., Massa, M., Robbioni, P., Ravelli, A., Burgio, G.R. and Martini, A. (1991) Correlation of serum interleukin-6 levels with joint involvement and thrombocytosis in systemic juvenile rheumatoid arthritis. *Arthritis Rheum*, **34**, 1158-1163.
- 14 Ahluwalia, T.S., Kilpelainen, T.O., Singh, S. and Rossing, P. (2019) Editorial: Novel Biomarkers for Type 2 Diabetes. *Front Endocrinol (Lausanne)*, **10**, 649.
- 15 Cesari, M., Penninx, B.W., Newman, A.B., Kritchevsky, S.B., Nicklas, B.J., Sutton-Tyrrell, K., Rubin, S.M., Ding, J., Simonsick, E.M., Harris, T.B. *et al.* (2003) Inflammatory markers and onset of cardiovascular events: results from the Health ABC study. *Circulation*, **108**, 2317-2322.

- 16 Qu, D., Liu, J., Lau, C.W. and Huang, Y. (2014) IL-6 in diabetes and cardiovascular complications. *Br J Pharmacol*, **171**, 3595-3603.
- 17 Mauer, J., Denson, J.L. and Bruning, J.C. (2015) Versatile functions for IL-6 in metabolism and cancer. *Trends Immunol*, **36**, 92-101.
- 18 Mucha, S., Baurecht, H., Novak, N., Rodriguez, E., Bej, S., Mayr, G., Emmert, H., Stolz, D., Gerdes, S., Jung, E.S. *et al.* (2020) Protein-coding variants contribute to the risk of atopic dermatitis and skin-specific gene expression. *J Allergy Clin Immunol*, **145**, 1208-1218.
- 19 Zhang, C., Wu, Z., Zhao, G., Wang, F. and Fang, Y. (2016) Identification of IL6 as a susceptibility gene for major depressive disorder. *Sci Rep*, **6**, 31264.
- 20 Calabrese, L.H. and Rose-John, S. (2014) IL-6 biology: implications for clinical targeting in rheumatic disease. *Nat Rev Rheumatol*, **10**, 720-727.
- 21 Scheinecker, C., Smolen, J., Yasothan, U., Stoll, J. and Kirkpatrick, P. (2009) Tocilizumab. *Nat Rev Drug Discov*, **8**, 273-274.
- 22 Revez, J.A., Bain, L.M., Watson, R.M., Towers, M., Collins, T., Killian, K.J., O'Byrne, P.M., Gauvreau, G.M., Upham, J.W. and Ferreira, M.A. (2019) Effects of interleukin-6 receptor blockade on allergen-induced airway responses in mild asthmatics. *Clin Transl Immunology*, **8**, e1044.
- 23 Yazici, Y., Curtis, J.R., Ince, A., Baraf, H., Malamet, R.L., Teng, L.L. and Kavanaugh, A. (2012) Efficacy of tocilizumab in patients with moderate to severe active rheumatoid arthritis and a previous inadequate response to disease-modifying antirheumatic drugs: the ROSE study. *Ann Rheum Dis*, **71**, 198-205.
- 24 Yokota, S., Imagawa, T., Mori, M., Miyamae, T., Aihara, Y., Takei, S., Iwata, N., Umebayashi, H., Murata, T., Miyoshi, M. *et al.* (2008) Efficacy and safety of tocilizumab in patients with systemic-onset juvenile idiopathic arthritis: a randomised, double-blind, placebo-controlled, withdrawal phase III trial. *Lancet*, **371**, 998-1006.
- 25 Gupta, S., Wang, W., Hayek, S.S., Chan, L., Mathews, K.S., Melamed, M.L., Brenner, S.K., Leonberg-Yoo, A., Schenck, E.J., Radbel, J. *et al.* (2020) Association Between Early Treatment With Tocilizumab and Mortality Among Critically Ill Patients With COVID-19. *JAMA Intern Med*, in press.
- 26 Worns, M.A., Victor, A., Galle, P.R. and Hohler, T. (2006) Genetic and environmental contributions to plasma C-reactive protein and interleukin-6 levels--a study in twins. *Genes Immun*, **7**, 600-605.
- 27 Sas, A.A., Jamshidi, Y., Zheng, D., Wu, T., Korf, J., Alizadeh, B.Z., Spector, T.D. and Snieder, H. (2012) The age-dependency of genetic and environmental influences on serum cytokine levels: a twin study. *Cytokine*, **60**, 108-113.
- 28 Neijts, M., van Dongen, J., Kluft, C., Boomsma, D.I., Willemsen, G. and de Geus, E.J. (2013) Genetic architecture of the pro-inflammatory state in an extended twin-family design. *Twin Res Hum Genet*, **16**, 931-940.
- 29 Amaral, W.Z., Krueger, R.F., Ryff, C.D. and Coe, C.L. (2015) Genetic and environmental determinants of population variation in interleukin-6, its soluble receptor and C-reactive protein: insights from identical and fraternal twins. *Brain Behav Immun*, **49**, 171-181.
- 30 Melzer, D., Perry, J.R., Hernandez, D., Corsi, A.M., Stevens, K., Rafferty, I., Lauretani, F., Murray, A., Gibbs, J.R., Paolisso, G. *et al.* (2008) A genome-wide association study identifies protein quantitative trait loci (pQTLs). *PLoS Genet*, **4**, e1000072.

- 31 Naitza, S., Porcu, E., Steri, M., Taub, D.D., Mulas, A., Xiao, X., Strait, J., Dei, M., Lai, S., Busonero, F. *et al.* (2012) A genome-wide association scan on the levels of markers of inflammation in Sardinians reveals associations that underpin its complex regulation. *PLoS Genet*, **8**, e1002480.
- 32 Comuzzie, A.G., Cole, S.A., Laston, S.L., Voruganti, V.S., Haack, K., Gibbs, R.A. and Butte, N.F. (2012) Novel genetic loci identified for the pathophysiology of childhood obesity in the Hispanic population. *PLoS One*, **7**, e51954.
- 33 Shah, T., Zabaneh, D., Gaunt, T., Swerdlow, D.I., Shah, S., Talmud, P.J., Day, I.N., Whittaker, J., Holmes, M.V., Sofat, R. *et al.* (2013) Gene-centric analysis identifies variants associated with interleukin-6 levels and shared pathways with other inflammation markers. *Circ Cardiovasc Genet*, **6**, 163-170.
- 34 Sidore, C., Busonero, F., Maschio, A., Porcu, E., Naitza, S., Zoledziewska, M., Mulas, A., Pistis, G., Steri, M., Danjou, F. *et al.* (2015) Genome sequencing elucidates Sardinian genetic architecture and augments association analyses for lipid and blood inflammatory markers. *Nat Genet*, **47**, 1272-1281.
- 35 Denny, J.C., Ritchie, M.D., Basford, M.A., Pulley, J.M., Bastarache, L., Brown-Gentry, K., Wang, D., Masys, D.R., Roden, D.M. and Crawford, D.C. (2010) PheWAS: demonstrating the feasibility of a phenome-wide scan to discover gene-disease associations. *Bioinformatics*, **26**, 1205-1210.
- 36 Pendergrass, S.A., Brown-Gentry, K., Dudek, S.M., Torstenson, E.S., Ambite, J.L., Avery, C.L., Buyske, S., Cai, C., Fesinmeyer, M.D., Haiman, C. *et al.* (2011) The use of phenome-wide association studies (PheWAS) for exploration of novel genotype-phenotype relationships and pleiotropy discovery. *Genet Epidemiol*, **35**, 410-422.
- 37 Pendergrass, S.A., Brown-Gentry, K., Dudek, S., Frase, A., Torstenson, E.S., Goodloe, R., Ambite, J.L., Avery, C.L., Buyske, S., Buzkova, P. *et al.* (2013) Phenome-wide association study (PheWAS) for detection of pleiotropy within the Population Architecture using Genomics and Epidemiology (PAGE) Network. *PLoS Genet*, **9**, e1003087.
- 38 So, H.C., Li, M. and Sham, P.C. (2011) Uncovering the total heritability explained by all true susceptibility variants in a genome-wide association study. *Genet Epidemiol*, **35**, 447-456.
- 39 Rentzsch, P., Witten, D., Cooper, G.M., Shendure, J. and Kircher, M. (2019) CADD: predicting the deleteriousness of variants throughout the human genome. *Nucleic Acids Res*, **47**, D886-D894.
- 40 Sabater-Lleal, M., Huang, J., Chasman, D., Naitza, S., Dehghan, A., Johnson, A.D., Teumer, A., Reiner, A.P., Folkersen, L., Basu, S. *et al.* (2013) Multiethnic meta-analysis of genome-wide association studies in >100 000 subjects identifies 23 fibrinogen-associated Loci but no strong evidence of a causal association between circulating fibrinogen and cardiovascular disease. *Circulation*, **128**, 1310-1324.
- 41 Dehghan, A., Dupuis, J., Barbalic, M., Bis, J.C., Eiriksdottir, G., Lu, C., Pellikka, N., Wallaschofski, H., Kettunen, J., Henneman, P. *et al.* (2011) Meta-analysis of genome-wide association studies in >80 000 subjects identifies multiple loci for C-reactive protein levels. *Circulation*, **123**, 731-738.
- 42 Okada, Y., Wu, D., Trynka, G., Raj, T., Terao, C., Ikari, K., Kochi, Y., Ohmura, K., Suzuki, A., Yoshida, S. *et al.* (2014) Genetics of rheumatoid arthritis contributes to biology and drug discovery. *Nature*, **506**, 376-381.

- 43 Yu, X.Q., Li, M., Zhang, H., Low, H.Q., Wei, X., Wang, J.Q., Sun, L.D., Sim, K.S., Li, Y., Foo, J.N. *et al.* (2011) A genome-wide association study in Han Chinese identifies multiple susceptibility loci for IgA nephropathy. *Nat Genet*, **44**, 178-182.
- 44 Liu, J.Z., van Sommeren, S., Huang, H., Ng, S.C., Alberts, R., Takahashi, A., Ripke, S., Lee, J.C., Jostins, L., Shah, T. *et al.* (2015) Association analyses identify 38 susceptibility loci for inflammatory bowel disease and highlight shared genetic risk across populations. *Nat Genet*, **47**, 979-986.
- 45 Elliott, P., Chambers, J.C., Zhang, W., Clarke, R., Hopewell, J.C., Peden, J.F., Erdmann, J., Braund, P., Engert, J.C., Bennett, D. *et al.* (2009) Genetic Loci associated with C-reactive protein levels and risk of coronary heart disease. *JAMA*, **302**, 37-48.
- 46 Ferreira, R.C., Freitag, D.F., Cutler, A.J., Howson, J.M., Rainbow, D.B., Smyth, D.J., Kaptoge, S., Clarke, P., Boreham, C., Coulson, R.M. *et al.* (2013) Functional IL6R 358Ala allele impairs classical IL-6 receptor signaling and influences risk of diverse inflammatory diseases. *PLoS Genet*, **9**, e1003444.
- 47 Stone, K., Woods, E., Szmania, S.M., Stephens, O.W., Garg, T.K., Barlogie, B., Shaughnessy, J.D., Jr., Hall, B., Reddy, M., Hoering, A. *et al.* (2013) Interleukin-6 receptor polymorphism is prevalent in HIV-negative Castleman Disease and is associated with increased soluble interleukin-6 receptor levels. *PLoS One*, **8**, e54610.
- 48 Rafiq, S., Frayling, T.M., Murray, A., Hurst, A., Stevens, K., Weedon, M.N., Henley, W., Ferrucci, L., Bandinelli, S., Corsi, A.M. *et al.* (2007) A common variant of the interleukin 6 receptor (IL-6r) gene increases IL-6r and IL-6 levels, without other inflammatory effects. *Genes Immun*, **8**, 552-559.
- 49 Galicia, J.C., Tai, H., Komatsu, Y., Shimada, Y., Akazawa, K. and Yoshie, H. (2004) Polymorphisms in the IL-6 receptor (IL-6R) gene: strong evidence that serum levels of soluble IL-6R are genetically influenced. *Genes Immun*, **5**, 513-516.
- 50 Hayashida, K., Bartlett, A.H., Chen, Y. and Park, P.W. (2010) Molecular and cellular mechanisms of ectodomain shedding. *Anat Rec (Hoboken)*, **293**, 925-937.
- 51 Collaboration, I.R.G.C.E.R.F., Sarwar, N., Butterworth, A.S., Freitag, D.F., Gregson, J., Willeit, P., Gorman, D.N., Gao, P., Saleheen, D., Rendon, A. *et al.* (2012) Interleukin-6 receptor pathways in coronary heart disease: a collaborative meta-analysis of 82 studies. *Lancet*, **379**, 1205-1213.
- 52 Interleukin-6 Receptor Mendelian Randomisation Analysis, C., Swerdlow, D.I., Holmes, M.V., Kuchenbaecker, K.B., Engmann, J.E., Shah, T., Sofat, R., Guo, Y., Chung, C., Peasey, A. *et al.* (2012) The interleukin-6 receptor as a target for prevention of coronary heart disease: a mendelian randomisation analysis. *Lancet*, **379**, 1214-1224.
- 53 Consortium, C.A.D., Deloukas, P., Kanoni, S., Willenborg, C., Farrall, M., Assimes, T.L., Thompson, J.R., Ingelsson, E., Saleheen, D., Erdmann, J. *et al.* (2013) Large-scale association analysis identifies new risk loci for coronary artery disease. *Nat Genet*, **45**, 25-33.
- 54 Schnabel, R.B., Kerr, K.F., Lubitz, S.A., Alkylbekova, E.L., Marcus, G.M., Sinner, M.F., Magnani, J.W., Wolf, P.A., Deo, R., Lloyd-Jones, D.M. *et al.* (2011) Large-scale candidate gene analysis in whites and African Americans identifies IL6R polymorphism in relation to atrial fibrillation: the National Heart, Lung, and

- Blood Institute's Candidate Gene Association Resource (CARE) project. *Circ Cardiovasc Genet*, **4**, 557-564.
- 55 Hawkins, G.A., Robinson, M.B., Hastie, A.T., Li, X., Li, H., Moore, W.C., Howard, T.D., Busse, W.W., Erzurum, S.C., Wenzel, S.E. *et al.* (2012) The IL6R variation Asp(358)Ala is a potential modifier of lung function in subjects with asthma. *J Allergy Clin Immunol*, **130**, 510-515 e511.
- 56 Harrison, S.C., Smith, A.J., Jones, G.T., Swerdlow, D.I., Rampuri, R., Bown, M.J., Aneurysm, C., Folkersen, L., Baas, A.F., de Borst, G.J. *et al.* (2013) Interleukin-6 receptor pathways in abdominal aortic aneurysm. *Eur Heart J*, **34**, 3707-3716.
- 57 Eyre, S., Bowes, J., Diogo, D., Lee, A., Barton, A., Martin, P., Zhernakova, A., Stahl, E., Viatte, S., McAllister, K. *et al.* (2012) High-density genetic mapping identifies new susceptibility loci for rheumatoid arthritis. *Nat Genet*, **44**, 1336-1340.
- 58 Scheller, J., Ohnesorge, N. and Rose-John, S. (2006) Interleukin-6 trans-signalling in chronic inflammation and cancer. *Scand J Immunol*, **63**, 321-329.
- 59 Chalaris, A., Schmidt-Arras, D., Yamamoto, K. and Rose-John, S. (2012) Interleukin-6 trans-signaling and colonic cancer associated with inflammatory bowel disease. *Dig Dis*, **30**, 492-499.
- 60 Enevold, C., Baslund, B., Linde, L., Josephsen, N.L., Tarp, U., Lindegaard, H., Jacobsen, S. and Nielsen, C.H. (2014) Interleukin-6-receptor polymorphisms rs12083537, rs2228145, and rs4329505 as predictors of response to tocilizumab in rheumatoid arthritis. *Pharmacogenet Genomics*, **24**, 401-405.
- 61 Ligthart, S., Vaez, A., Vosa, U., Stathopoulou, M.G., de Vries, P.S., Prins, B.P., Van der Most, P.J., Tanaka, T., Naderi, E., Rose, L.M. *et al.* (2018) Genome Analyses of >200,000 Individuals Identify 58 Loci for Chronic Inflammation and Highlight Pathways that Link Inflammation and Complex Disorders. *Am J Hum Genet*, **103**, 691-706.
- 62 Reiner, A.P., Beleza, S., Franceschini, N., Auer, P.L., Robinson, J.G., Kooperberg, C., Peters, U. and Tang, H. (2012) Genome-wide association and population genetic analysis of C-reactive protein in African American and Hispanic American women. *Am J Hum Genet*, **91**, 502-512.
- 63 Herder, C., Nuotio, M.L., Shah, S., Blankenberg, S., Brunner, E.J., Carstensen, M., Gieger, C., Grallert, H., Jula, A., Kahonen, M. *et al.* (2014) Genetic determinants of circulating interleukin-1 receptor antagonist levels and their association with glycemic traits. *Diabetes*, **63**, 4343-4359.
- 64 Ellingsgaard, H., Hauselmann, I., Schuler, B., Habib, A.M., Baggio, L.L., Meier, D.T., Eppler, E., Bouzakri, K., Wueest, S., Muller, Y.D. *et al.* (2011) Interleukin-6 enhances insulin secretion by increasing glucagon-like peptide-1 secretion from L cells and alpha cells. *Nat Med*, **17**, 1481-1489.
- 65 van de Veerdonk, F.L., Stoeckman, A.K., Wu, G., Boeckermann, A.N., Azam, T., Netea, M.G., Joosten, L.A., van der Meer, J.W., Hao, R., Kalabokis, V. *et al.* (2012) IL-38 binds to the IL-36 receptor and has biological effects on immune cells similar to IL-36 receptor antagonist. *Proc Natl Acad Sci U S A*, **109**, 3001-3005.
- 66 Reiner, A.P., Wurfel, M.M., Lange, L.A., Carlson, C.S., Nord, A.S., Carty, C.L., Rieder, M.J., Desmarais, C., Jenny, N.S., Iribarren, C. *et al.* (2008) Polymorphisms of the IL1-receptor antagonist gene (IL1RN) are associated with multiple markers of systemic inflammation. *Arterioscler Thromb Vasc Biol*, **28**, 1407-1412.
- 67 Hom, G., Graham, R.R., Modrek, B., Taylor, K.E., Ortmann, W., Garnier, S., Lee, A.T., Chung, S.A., Ferreira, R.C., Pant, P.V. *et al.* (2008) Association of

systemic lupus erythematosus with C8orf13-BLK and ITGAM-ITGAX. *N Engl J Med*, **358**, 900-909.

68 Jostins, L., Ripke, S., Weersma, R.K., Duerr, R.H., McGovern, D.P., Hui, K.Y., Lee, J.C., Schumm, L.P., Sharma, Y., Anderson, C.A. *et al.* (2012) Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. *Nature*, **491**, 119-124.

69 Ahluwalia, T.S., Schulz, C.A., Waage, J., Skaaby, T., Sandholm, N., van Zuydam, N., Charmet, R., Bork-Jensen, J., Almgren, P., Thuesen, B.H. *et al.* (2019) A novel rare CUBN variant and three additional genes identified in Europeans with and without diabetes: results from an exome-wide association study of albuminuria. *Diabetologia*, **62**, 292-305.

70 Apelqvist, J. (2012) Diagnostics and treatment of the diabetic foot. *Endocrine*, **41**, 384-397.

71 Pickrell, J.K., Berisa, T., Liu, J.Z., Segurel, L., Tung, J.Y. and Hinds, D.A. (2016) Detection and interpretation of shared genetic influences on 42 human traits. *Nat Genet*, **48**, 709-717.

72 MacRae, C.A. and Vasan, R.S. (2011) Next-generation genome-wide association studies: time to focus on phenotype? *Circ Cardiovasc Genet*, **4**, 334-336.

73 Dumitrescu, L., Carty, C.L., Franceschini, N., Hindorff, L.A., Cole, S.A., Buzkova, P., Schumacher, F.R., Eaton, C.B., Goodloe, R.J., Duggan, D.J. *et al.* (2013) Post-genome-wide association study challenges for lipid traits: describing age as a modifier of gene-lipid associations in the Population Architecture using Genomics and Epidemiology (PAGE) study. *Ann Hum Genet*, **77**, 416-425.

74 Kulminski, A.M., Culminskaya, I., Arbeev, K.G., Arbeeva, L., Ukraintseva, S.V., Stallard, E., Wu, D. and Yashin, A.I. (2015) Birth Cohort, Age, and Sex Strongly Modulate Effects of Lipid Risk Alleles Identified in Genome-Wide Association Studies. *PLoS One*, **10**, e0136319.

75 Marchini, J., Howie, B., Myers, S., McVean, G. and Donnelly, P. (2007) A new multipoint method for genome-wide association studies by imputation of genotypes. *Nat Genet*, **39**, 906-913.

76 Howie, B., Fuchsberger, C., Stephens, M., Marchini, J. and Abecasis, G.R. (2012) Fast and accurate genotype imputation in genome-wide association studies through pre-phasing. *Nat Genet*, **44**, 955-959.

77 Servin, B. and Stephens, M. (2007) Imputation-based analysis of association studies: candidate regions and quantitative traits. *PLoS Genet*, **3**, e114.

78 van der Most, P.J., Vaez, A., Prins, B.P., Munoz, M.L., Snieder, H., Alizadeh, B.Z. and Nolte, I.M. (2014) QCGWAS: A flexible R package for automated quality control of genome-wide association results. *Bioinformatics*, **30**, 1185-1186.

79 Magi, R. and Morris, A.P. (2010) GWAMA: software for genome-wide association meta-analysis. *BMC Bioinformatics*, **11**, 288.

80 Pei, Y.F., Zhang, L., Li, J. and Deng, H.W. (2010) Analyses and comparison of imputation-based association methods. *PLoS One*, **5**, e10827.

81 Willer, C.J., Li, Y. and Abecasis, G.R. (2010) METAL: fast and efficient meta-analysis of genomewide association scans. *Bioinformatics*, **26**, 2190-2191.

82 Yang, J., Benyamin, B., McEvoy, B.P., Gordon, S., Henders, A.K., Nyholt, D.R., Madden, P.A., Heath, A.C., Martin, N.G., Montgomery, G.W. *et al.* (2010) Common

SNPs explain a large proportion of the heritability for human height. *Nat Genet*, **42**, 565-569.

83 Penninx, B.W., Beekman, A.T., Smit, J.H., Zitman, F.G., Nolen, W.A., Spinhoven, P., Cuijpers, P., De Jong, P.J., Van Marwijk, H.W., Assendelft, W.J. *et al.* (2008) The Netherlands Study of Depression and Anxiety (NESDA): rationale, objectives and methods. *Int J Methods Psychiatr Res*, **17**, 121-140.

84 Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A., Bender, D., Maller, J., Sklar, P., de Bakker, P.I., Daly, M.J. *et al.* (2007) PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet*, **81**, 559-575.

85 Hinrichs, A.S., Karolchik, D., Baertsch, R., Barber, G.P., Bejerano, G., Clawson, H., Diekhans, M., Furey, T.S., Harte, R.A., Hsu, F. *et al.* (2006) The UCSC Genome Browser Database: update 2006. *Nucleic Acids Res*, **34**, D590-598.

86 Danecek, P., Auton, A., Abecasis, G., Albers, C.A., Banks, E., DePristo, M.A., Handsaker, R.E., Lunter, G., Marth, G.T., Sherry, S.T. *et al.* (2011) The variant call format and VCFtools. *Bioinformatics*, **27**, 2156-2158.

87 Wang, K., Li, M. and Hakonarson, H. (2010) ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Res*, **38**, e164.

88 Pruitt, K.D., Tatusova, T. and Maglott, D.R. (2005) NCBI Reference Sequence (RefSeq): a curated non-redundant sequence database of genomes, transcripts and proteins. *Nucleic Acids Res*, **33**, D501-504.

89 Staley, J.R., Blackshaw, J., Kamat, M.A., Ellis, S., Surendran, P., Sun, B.B., Paul, D.S., Freitag, D., Burgess, S., Danesh, J. *et al.* (2016) PhenoScanner: a database of human genotype-phenotype associations. *Bioinformatics*, **32**, 3207-3209.

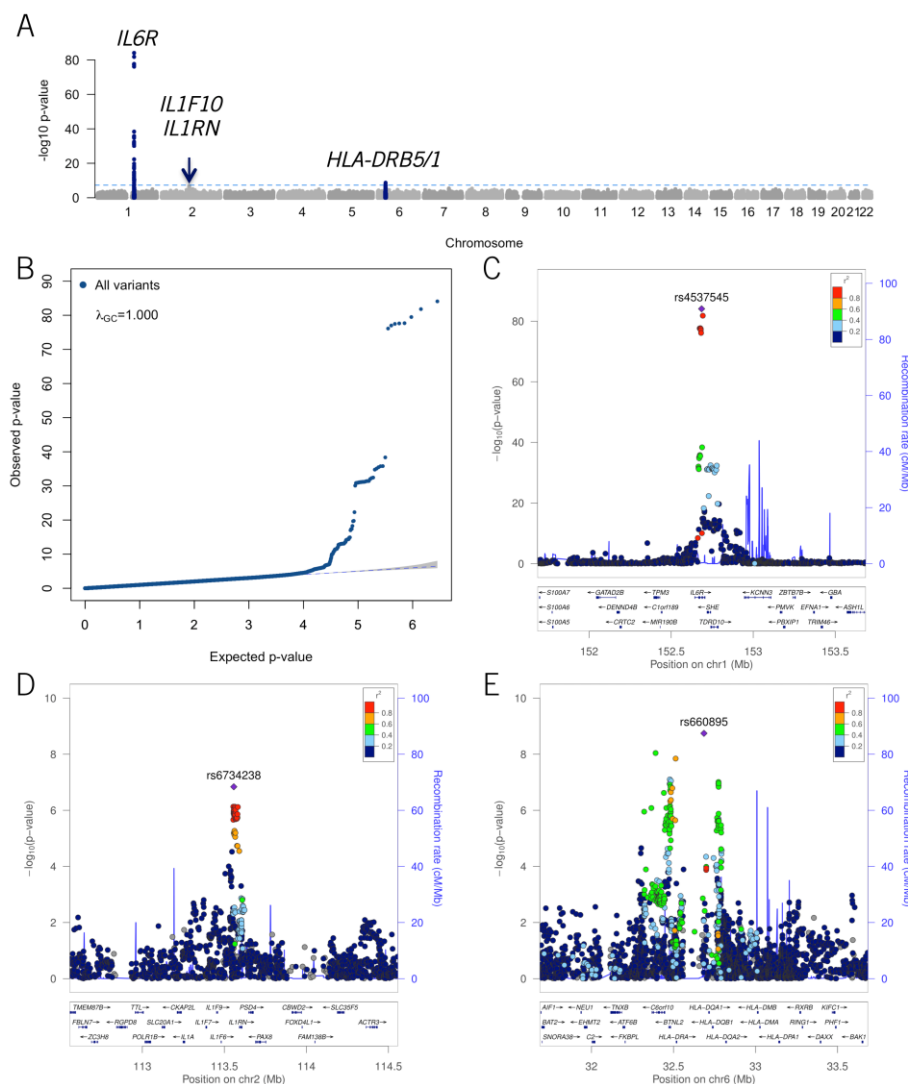
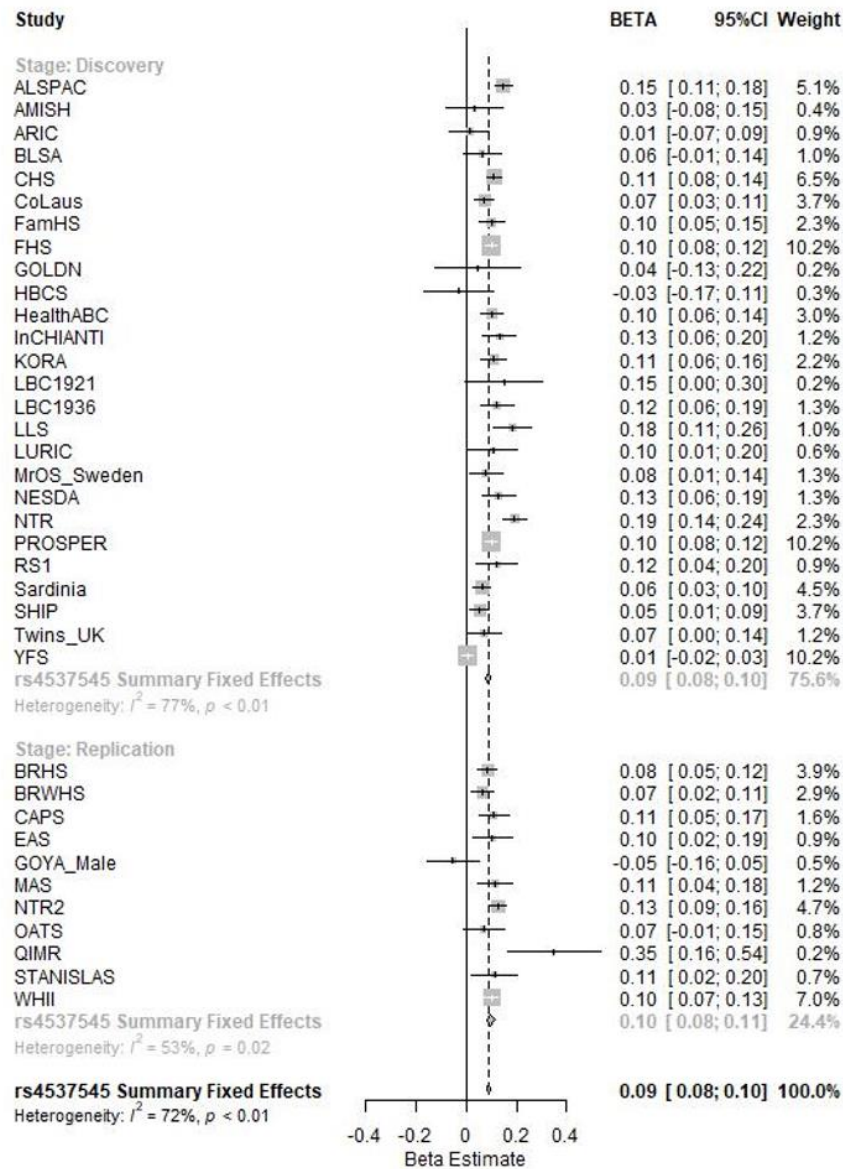
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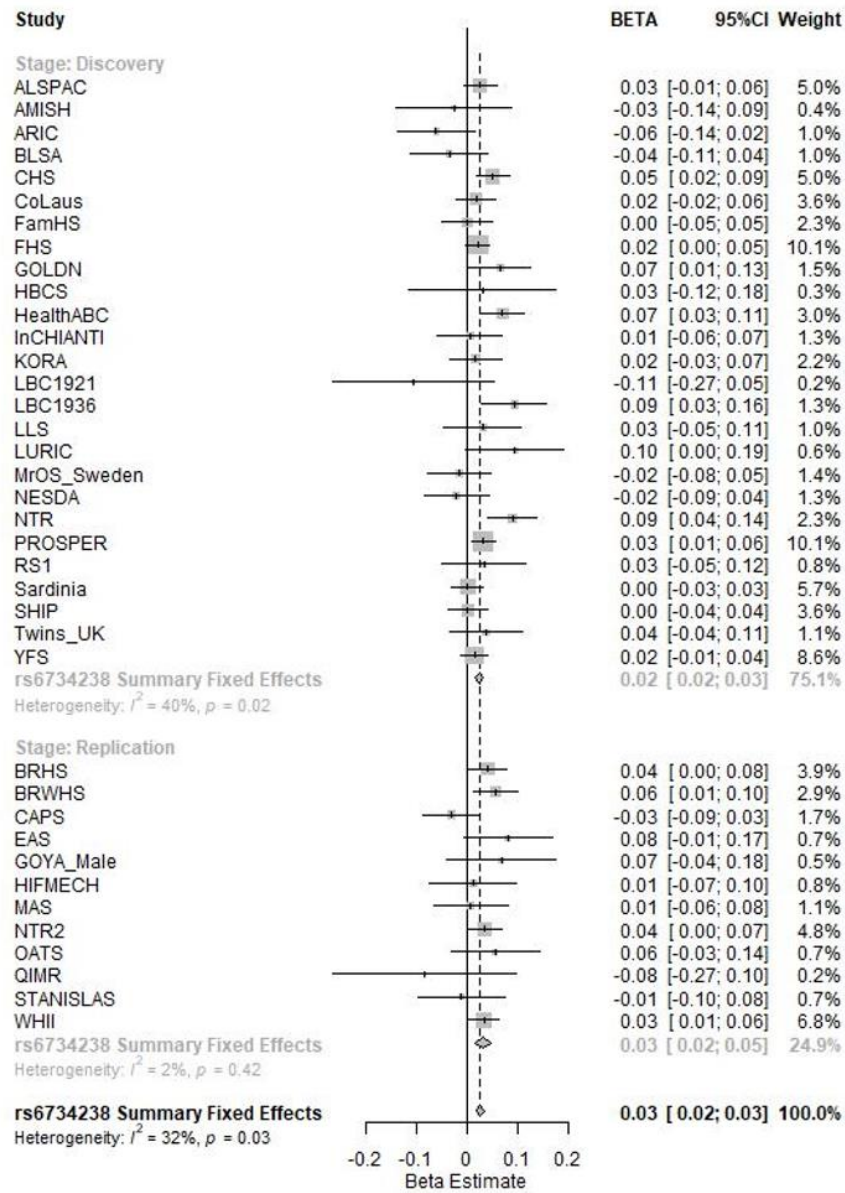
Figure 1.
Manhattan, QQ and LocusZoom plots of the discovery GWAS meta-analyses.

A) Manhattan plot showing the association of SNPs with IL-6. Loci coloured in red or blue, three in total, represent those for which the lead SNPs reached genome-wide significance ($P=5 \times 10^{-8}$). Horizontal axis: relative genomic position of variants on the genome, vertical axis: $-\log_{10}$ p-value of each SNP; **B)** Quantile-quantile plot for p-values obtained from the meta-analysis. The horizontal and vertical axes represents the expected distribution of $-\log_{10}$ (P-values) under the null hypothesis of no association, whereas the vertical axis shows the observed $-\log_{10}$ (P-values). The blue dashed line represents the null, and λ_{GC} value represents the genomic inflation factor lambda. Each data point represents the observed versus the expected p-value of a variant included in the association analyses; **C-E)** Regional association plots for each of the three genome-wide significant loci, 1q21, 2q14, and 6p21, respectively. Pairwise LD (r^2) with the lead SNP is indicated following a color-coded scale. Horizontal axis: relative genomic position of variants within the locus, vertical axis: $-\log_{10}$ p-value of each SNP.

A.



B.



C.

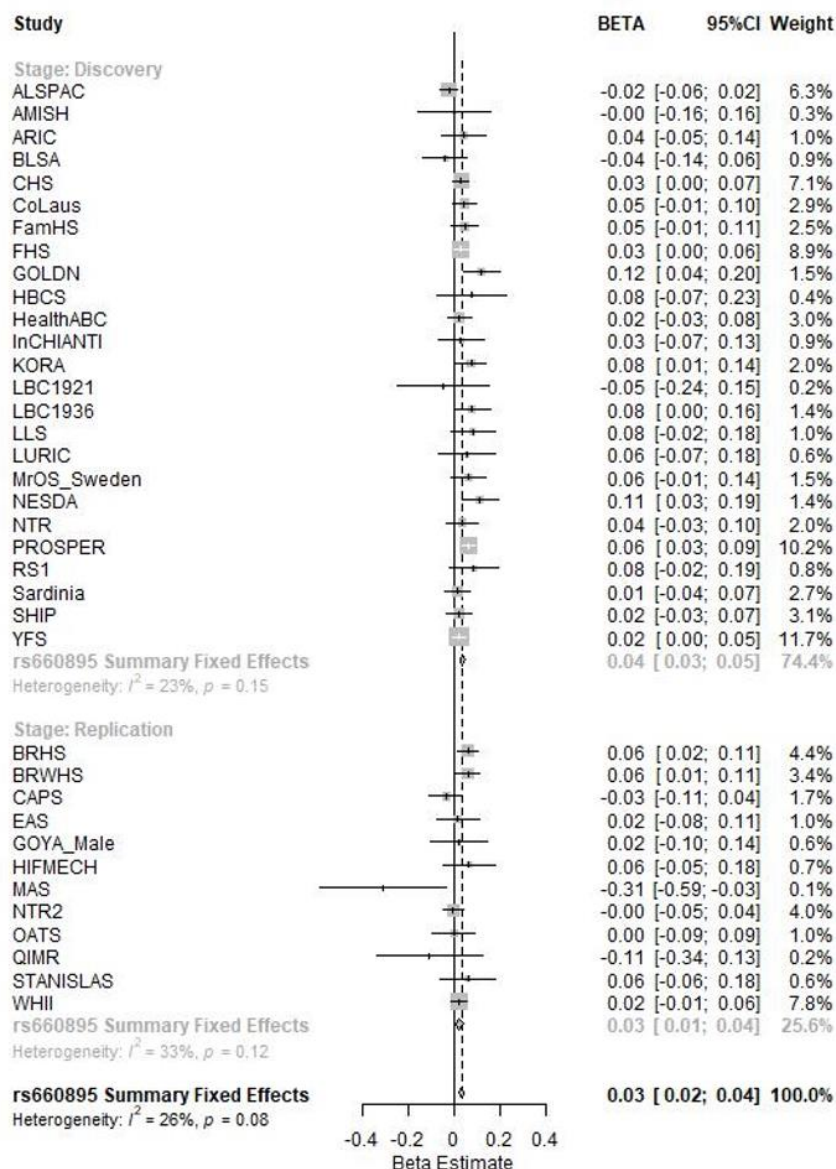


Figure 2. Combined discovery and replication forest plots for the GWAS Index SNPs. Forest plots for A) *IL6R* rs4537545 (chr. 1q21), B) *IL1RN* rs6734238 (chr. 2q14), C) *HLA-DRB5* rs660895 (chr. 6p21) with discovery, replication and combined effect estimates, 95%CI and weights based on the fixed effects inverse variance meta-analyses.

Table 1: Novel and replicated loci associated with circulating IL-6 levels at $p < 5.0 \times 10^{-8}$ in the combined GWAS meta analyses

Chr	Lead SNP (rsID)	BP (Hg19)	Effect/Other allele	EA F	Beta (SE)	$P_{\text{discovery}}$	$P_{\text{replication}}$	P_{combined}	Annotation	Nearest Genes	$P_{\text{het}} (I^2)$
<i>Novel Loci</i>											
2q14	rs6734238	113841030	G/A	0.42	0.025 (0.005)	1.45×10^{-7}	3.24×10^{-5}	1.84×10^{-11}	intergenic	<i>IL1F10/IL1RN</i>	0.03 (32%)
6p21	rs660895	32577380	G/A	0.19	0.036 (0.006)	1.80×10^{-9}	3.38×10^{-2}	1.55×10^{-10}	intergenic	<i>HLA-DRB5/DRB1</i>	0.08 (26%)
<i>Replicated Known Locus</i>											
1q21	rs4537545	154418879	T/C	0.39	0.091 (0.005)	8.39×10^{-85}	7.88×10^{-37}	1.20×10^{-122}	intronic	<i>IL6R</i>	< 0.01 (75%)

Index SNPs that reached $p < 5 \times 10^{-8}$ in the combined analysis from each locus are reported here. Sample sizes: discovery cohorts, $n=52,654$; replication cohorts, $n=14,774$; combined, $n=67,428$. The effect sizes (β) in the discovery phase, given for the effect allele. EAF: Effect Allele Frequency; Effect sizes and standard error (SE) values are based on natural log transformed IL6 (pg/ml) levels. Phet: Combined Meta-analysis heterogeneity P value; I2: Heterogeneity measure